REMARKS

I. ELECTION/RESTRICTIONS

A. Claims 13, 14, 24, 25 and 32-42

The Examiner states "claims 13, 14, 24, 25, 32-42 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a non-elected invention, there being no allowable generic or linking claim."

Applicants will cancel claims 13, 14, 24, 25 and 32-42 upon the allowance of claims in the above-identified application.

II. CLAIM REJECTIONS – 35 U.S.C. §102

A. Claims 1-9 and 15-22

Claims 1-9 and 15-22 were rejected under 35 U.S.C. 102(b) as being anticipated by Tanner et al. (U.S. Patent No. 5,714,377).

The Examiner states "Tanner et al. disclose a method of producing a heterologous protein in fungi, which is a yeast cell, comprising providing a yeast cell having a mutation in a PMT 1 gene, in which O-glycosylation is inhibited. The transformation may be the LiAc method or any other technique disclosed in the literature. Yeast based plasmid may be used."

Applicants have amended independent claim 1 to recite "providing a recipient fungi cell wherein the expression of PMT 2 is inhibited". Independent claim 15 has been amended similarly. Such is not disclosed by Tanner et al. Applicants respectively submit Tanner discloses that the modified fungal cells carrying genetic modification within the PMT 1 gene causes the cell to have at least a reduced capacity for O-glycosylating foreign proteins (col. 2, lines 52-56). Tanner fails to disclose or recognize that modification of a PMT 2 gene and wherein said modification results in incompletely or misfolded folded heterologous proteins to not be

degraded in the endoplasmic reticulum and wherein the inhibition enhances folding and assembly of said heterologous proteins. Further, Applicants submit dependent claims 2-6 and 16-20, by virtue of their dependency, contain all the limitations to amended independent claims 1 and 15, respectively.

Further, Applicants submit the limitations of the amended claims are not obvious in view of Tanner et al. Applicants are herein submitting a declaration under 37 CFR 1.132 of Dr. Davis Ng, a co-inventor, wherein Dr. Ng demonstrates that the folding of KGF, a reporter construct, is enhanced in PMT 2 mutant yeast cells and yeast cells possessing double disruption of the PMT 1/PMT 2 genes, but not with PMT 1 inhibition. Moreover, the declaration provides clear evidence that the invention is beneficial for increasing the folding/assembly of a second molecule, humanized monoclonal antibodies, which is more complex than the green fluorescent protein. Therefore, it would not be obvious to one of ordinary skill in the art to modify the teachings of Tanner and arrive at Applicants' claimed invention. As shown in Exhibit B, the PMT 1 mutant emitted less fluorescence, thereby indicating its inhibition had little benefit to improving folding over both a PMT 2 mutant and a PMT 1/ PMT 2 double mutant in yeast cells. Therefore, Applicants respectfully request this rejection be withdrawn.

B. Claims 1, 2, 5, 6, 7, 15, 16, 19 and 20

Claims 1, 2, 5, 6, 7, 15, 16, 19 and 20 were rejected under 35 U.S.C. 102(b) as being anticipated by Ernst et al. (WO 94/26873).

The Examiner states "Ernst et al. disclose a method of producing a heterologous protein in fungi comprising providing a recipient cell in which O-glycosylation is inhibited, and introducing a polynucleotide expression construct. The polynucleotide may be within a yeast

based plasmid. The recipient cell has inhibited O-glycosylation activity, and the defect is in a protein mannosyltransferase."

Applicants have amended independent claim 1 to recite "providing a recipient fungi cell wherein the expression of PMT 2 is inhibited". Independent claim 15 has been amended similarly. Such is not disclosed by Ernst et al. Applicants submit Ernst et al. merely disclose a yeast strain that performs O-glycosylation of at least one serine and/or at least one threonine residue at a reduced level and methods of production of heterologous proteins. However, Ernst does not disclose PMT 2. Therefore, Ernst et al. does not anticipate.

Further, Applicants submit the limitations of the amended claims are not obvious in view of Ernst et al. As shown in the 132 declaration submitted herein, Applicants demonstrate that disruption of PMT 2 and PMT 1/PMT 2 together, can initiate Applicants' claimed characteristics. Ernst does not even mention protein folding and assembly nor that incompletely folded heterologous proteins are no longer degraded in the endoplasmic reticulum by such inhibition. In fact, Ernst discloses that for the mutants isolated in their study, they were not sure how the mutants became defected (see page 11, lines 14-15). Applicants respectfully request this rejection be withdrawn.

III. CLAIM REJECTIONS – 35 U.S.C. §103

A. Claims 1-7 and 15-20

Claims 1-7 and 15-20 were rejected under 35 U.S.C. §103(a) as being unpatentable over Ernst et al. (WO 94/26873) in view of Ito et al. (*J. Bacteriol.*, 153(1): 163-8 (1983)).

The Examiner states "it would have been obvious to one of ordinary skill in the art to use the well known LiAc transformation method disclosed by Ito et al. in the method of producing a heterologous protein, as disclosed by Ernst et al. because Ernst et al. teach that it is within the

ordinary skill in the art to introduce an expression construct into fungi to produce a heterologous protein and Ito et al. teach that it is within the ordinary skill in the art to transform yeast using LiAc."

Applicants respectfully traverse this rejection. There is no motivation and/or suggestion in the references that they be combined in the manner suggested by the Examiner. Absent such a suggestion, there would be no reason why one skilled in the art looking for a solution to the problem of providing a method of producing heterologous proteins in fungi using yeast, without the protein misfolding problems, as exhibited by Applicants, would consult the particular combination of references suggested by the Examiner.

Ernst et al. teach a *S. Cerevisiae* strain that is defective in O-mannosylation of the hydroxyl group of at least one residue selected from serine and threonine in a protein expressed by the strain. Applicants respectfully submit Ernst discloses that they are not sure where the defect is. Specifically, they state, "this is probably due to a defect in the recognition of serine or threonine acceptor sites, or in the mannosylation transferase activity required to glycoslylate these sites" (See page 3 starting at line 37 to page 4, line 2). Therefore, they fail to recognize or appreciate that modifying the PMT 2 gene allows incompletely folded heterologous proteins to not be degraded in the endoplasmic reticulum and wherein such modification in the cell enhances protein folding, thereby facilitating proper synthesis of heterologous proteins.

Ito is directed at developing a more convenient method for yeast transformation. Ito teaches that intact yeast cells treated with alkali cations take up more plasmid DNA, wherein one of the cations disclosed is lithium (Li^+). Ito shows absolutely no recognition of, or pertinence, to the nature of the problem of producing heterologous protein in fungi; therefore, one skilled in the art would not be likely to use such a reference alone or in combination with Ernst in an

attempt to solve problems of inappropriate modification of heterologous proteins in fungi. Applicants submit that these references, alone and in combination, relate to different problems and therefore, a suggestion to combine is not indicated. See *In re Zurko*, 111 F.3d 887, 890 (Fed. Cir. 1997)(stating, "to say that the missing step comes from the nature of the problem to be solved begs the question because the Board has failed to show that this problem had been previously identified anywhere in the prior art"); *In re Wright*, 848 F.2d 1216 (Fed. Cir. 1988)(differences between the problem solved by the invention and those solved in the prior art may defeat the rejection). The Applicants' claimed features (incompletely folded proteins are not degraded in the endoplasmic reticulum and enhancing protein folding and assembly) are not found either of the references; therefore, such a feature would be lacking in any combination. Applicants respectfully request this rejection be withdrawn.

B. Claims 1-10 and 15-23

Claims 1-10 and 15-23 were rejected under 35 U.S.C. §103(a) as being unpatentable over Tanner et al. (U.S. Patent No. 5,714,277) in view of Strahl-Bolsinger et al. (*Biochem. Biophys. Acta* 1426: 297-307 (1999)).

The Examiner states "it would have been obvious to one of ordinary skill in the art to substitute for the yeast cell having a mutation in the PMT 1 gene in the method taught by Tanner et al., with a yeast cell having a mutation in any one of the PMT 2-6 genes as taught by Strahl-Bolsinger et al. because Tanner et al. teach that it is within the ordinary skill in the art to produce heterologous protein in yeast cells in which O-glycosylation is inhibited and Strahl-Bolsinger et al. teach that O-glycosylation can be inhibited by defects in any other PMT 1-6 genes."

Applicants respectfully traverse this rejection. Dr. Ng's declaration demonstrates that substituting a PMT gene and arriving at Applicants' claimed invention would not be obvious to

one of ordinary skill in the art because the data (Exhibit B) shows that a mutation in a PMT 1 gene does not necessarily enhance protein folding when compared to a yeast cell having a PMT 2 mutation or a PMT 1/PMT 2 double mutation. Moreover, this demonstrates unexpected superior results over what is disclosed by Tanner as well as Strahl-Bolsinger et al. alone, or in combination.

Additionally, Applicants submit there is no motivation and/or suggestion in the references that they be combined in the manner suggested by the Examiner. Absent such a suggestion, there would be no reason why one skilled in the art, who is faced with the same problem confronting the Applicants, i.e., providing a method of producing heterologous proteins in fungi using yeast, without the protein misfolding problems, and who had no prior knowledge of Applicants' claimed method, would consult the particular combination of references suggested by the Examiner. Tanner teaches fungal cells carrying a modification in PMT 1 causes the cells to exhibit at least a reduced capacity for O-glycosylating heterologous proteins. Strahl-Bolsinger teaches the main aspects of biogenesis of O-linked carbohydrate chains in *S. Cerevisiae*, using PMT mutants that demonstrate the impact of protein mannosylation on protein secretion, on maintenance of cell wall integrity and on budding. Both references fail to recognize previous problems associated with the yeast expression system where many of the heterologous proteins fail to fold (spec. at page 3 - Summary of the Invention, 1st para.). The problem solved by the present invention was never before even recognized. The recognition of an unrecognized problem militates in favor of patentability. Moreover, both references do not show an appreciation of the advantage or benefits of inhibiting PMT 2 and PMT 1/PMT 2 so that incompletely folded heterologous proteins are not degraded in the endoplasmic reticulum and enhancing folding and assembly of the these proteins, thus promoting proper protein synthesis.

That this has gone unrecognized for years by those skilled in this art argues in favor of nonobviousness. Therefore, one of ordinary skill in the art looking to solve the same problem as that of the Applicants would not look to this combination of references as proposed by the Examiner in an attempt to solve the problems confronted by Applicants. Therefore, this rejection must be withdrawn.

IV. CLAIM REJECTIONS – 35 U.S.C. §112

A. Claims 1-5

Claims 1-5 were rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement.

The Examiner states "claims 1-5 are genus claims in terms of a method using any recipient fungi cell wherein the cell has any modification in which incompletely folded heterologous proteins are not degraded in the endoplasmic reticulum." "[T]here is no disclosure of common structure possessed by fungi cells which would result in the lack of degradation of incompletely folded heterologous proteins. There is no structure/function analysis of the disclosed yeast PMT or BST mutant cells to provide guidance on the essential genes that could inhibit and result in the claimed characteristics."

Applicants have amended independent claim 1 to recite a gene (PMT 2) that could be inhibited and result in the claimed characteristics. Claims 2-5 by virtue of their dependency contain all the limitations of amended independent claim 1. Applicants respectfully request this rejection be withdrawn.

B. Claims 7-12, 15-23, and 26-31

Claims 7-12, 15-23, and 26-31 were rejected under 35 U.S.C. 112, second paragraph, as being indefinite.

The Examiner states "claim 7 and by dependence claims 7-12 are vague and indefinite in the recitation of recipient cell comprises inhibition of a protein mannosyltransferase gene. It is not clear what is intended by this phrase. The claim has been examined as if it recited: 'The method of claim 6 wherein said recipient cell is modified so that the expression of a protein mannosyltransferase gene is inhibited'".

Applicants have cancelled claims 7-12, thus making this rejection moot.

Next, the Examiner states "claim 11 recites the limitation 'said recipient gene' in line 1. There is insufficient antecedent basis for this limitation in the claim. It is noted that if one of the PMT 1-6 genes recited in claim 8 are intended as the 'recipient gene', it is not understood how it 'provides inhibition' of the Bypass Sec Thirteen gene since they encode protein mannosyltransferase, rather than a regulatory protein."

Applicants have cancelled claims 11-12, thus making this rejection moot.

The Examiner states "claim 26, and by dependence, claims 27-31 are rejected under 35 U.S.C. 112 second paragraph as being indefinite. Claims 15 and 26 are indefinite in its recitation of 'structural gene'. It is not known what is intended by this phrase."

Applicants have amended claims 15 and 26 by deleting the recitation "structural gene" and replacing it with the recitation "DNA sequence capable of being expressed in said cell", to more clearly recite that the construct comprises a DNA sequence of interest that is transcribed into mRNA and translated into a polypeptide (see spec. at page 13 – "structural gene"). Applicants respectfully request this rejection be withdrawn.

V. CONCLUSION

Please charge Deposit Account No. 26-0084 in the amount of $1314.00 to cover the cost of (a) newly added claims and (b) a one month extension ($55.00) from April 29, 2004 to May 29,

2004. No other fees or extensions of time are believed to be due in connection with this amendment; however, consider this a request for any extension inadvertently omitted, and charge any additional fees to Deposit Account No. 26-0084.

Reconsideration and allowance is respectfully requested.

Respectfully submitted,

HEIDI S. NEBEL, Reg. No. 37,719
McKEE, VOORHEES & SEASE, P.L.C.
801 Grand Avenue, Suite 3200
Des Moines, Iowa 50309-2721
Phone No: (515) 288-3667
Fax No: (515) 288-1338
CUSTOMER NO: 27407

Attorneys of Record

- mlw/tyb -